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(54) Title: LIPOSOME INHALATION METHOD AND SYSTEM

(57) Abstract

A method and system for administering a drug by inhalation. The system includes a suspension of liposomes containing the drug predominantly in a liposome-entrapped form, where the liposomes have a phospholipid composition selected to produce a desired drug-release rate when administered to the respiratory tract. The invention is useful in delivering a drug systematically, under controlled drug-release rates, as well as to a target site in the respiratory tract.

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LIPOSOME INHALATION METHOD AND SYSTEM

1. Field of the Invention

The present invention relates to drug delivery
by inhalation, and, in particular, to an improved drug
delivery system and method of using liposome-entrapped
drugs.

2. References

The following references are incorporated
herein by corresponding number:

1. Hollenbeck, R.G., et al, in "Pharmaceutics and
   Pharmacy Practice" (Banker, G.S., et al, eds), J.P.
   Lippincott, Philadelphia (1982), pp. 355-358 .

2. Szoka, F., Jr., et al, Ann Rev Biophys Bioeng
   (1980), 9:467.

3. Szoka, F., Jr., et al, Proc Natl Acad Sci (USA)
   (1978) 75:4194.

4. Hollenbeck, R.G., op cit, pp. 382-391.

3. Background and Summary

Inhalation provides an effective means for
delivering a variety of drugs, including nasal
decongestants, drugs useful in the treatment of asthma
and other bronchial and pulmonary conditions (reference
1). An obvious advantage of inhalation in treating
nasal, bronchial, or pulmonary conditions is the ability
to deliver the drug directly to the site of drug
action. A related advantage is the rapid onset of the
therapeutic effect, compared with other routes of
administration, such as intramuscular and oral routes.
For drugs which are susceptible to breakdown in the
gastrointestinal tract, or which otherwise cannot be
administered orally, inhalation may be preferred for a

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variety of reasons over intravenous or intramuscular
injection. Other drugs, such as nitroglycerin, whose
primary drug action is systemic, can also be delivered
efficiently by inhalation.

5   In one known method for administering a drug by
inhalation, the drug is dissolved in a suitable solvent
which can be aerosolized to form a small-particle mist.
The drug solution may be aerosolized by a pneumatic or
ultrasonic nebulizer, or, more conveniently, by a
10 self-contained nebulizer which is driven by gas pressure
from a fluorocarbon propellant. Inhalation of the
aerosolized mist, i.e., drawing the mist from the mouth
or nose into the respiratory tract, acts to deposit the
drug-containing aerosol particles on various sites of
15 the respiratory tract, including the upper
nasopharyngeal region, the tracheobronchial region, and
the pulmonary region. In the latter region, the drug
has the opportunity for rapid absorption into the
bloodstream for systemic action.

20   Also well known in the prior art are inhalation
systems in which a drug is administered in particulate
form, either as a dry powder or as a micronized
suspension in a suitable carrier solvent system.
Typically the drug is a water-soluble compound which is
25 suspended in micronized form in a fluorocarbon-type
propellant solvent. Following aerosolization, most of
the propellant solvent is lost through flash evaporation
and replaced by moisture in the respiratory tract,
leading to the deposition of hydrated micronized
30 particles.

    Both types of inhalation systems mentioned
above are based on delivery of the drug in a free form
to sites in the respiratory tract. As such, the drug is
rapidly utilized and, in the case of pulmonary

deposition, taken up systemically at the site of
deposition. Because of this rapid drug uptake and
utilization, it may be necessary to administer the drug
at frequent intervals to maintain a desired therapeutic
5   dose level at the site of action. A related problem is
the limitation on the amount of drug that can be
administered safely at each inhalation, particularly in
the case of a drug which has unwanted systemic side
effects. This problem is illustrated by a number of
10   β-adrenergic agonist type bronchodilators which also
produce marked tachycardia. Even at relatively low
doses of these drugs, the stimulatory effect of the drug
on the heart may be a nuisance to the patient. An
additional problem associated with administration of
15   micronized particles is the irritation that these
particles may produce in the respiratory tract.

More recently, a liposome inhalation system for
administering a drug to the respiratory tract in
liposome-entrapped form has been proposed (UK patent
20   application # 2,145,107A. A liposome aerosol is formed
by mixing lipid and aqueous components in a two-chamber
dispenser, then aerosolizing the mixture by forcing it
through a nozzle. The mixing and aerosolizing steps are
effective to form a liposome aerosol in which a portion
25   of the drug, which may be contained in either the lipid
or aqueous component, is present in liposome-entrapped
form. This invention suffers the disadvantage that _in
situ_ liposome formation, and particularly the
efficiency of drug encapsulation in the liposomes, is
30   highly variable, depending on the degree of mixing of
the two components just prior to aerosolization, the
relative quantities of two components which are mixed,
and possibly temperature and humidity conditions which
exist during liposome formation. As a result the

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relative percentages of free and liposome-encapsulated
drug, and even the total amount of drug which is
delivered can be quite variable.

5   4. Summary of the Invention
        It is a general object of the present invention
to provide a liposome drug inhalation method and system
which substantially overcome problems and limitations
associated with prior art devices and methods for
10 administering drugs by the inhalation route.
        One specific object of the invention is to
provide such method and system for use in delivering a
water-soluble, liposome-permeable drug, and particularly
a system which can be stored over a long period before
15 use.
        Still another object of the invention is to
provide a method and system in which the rate of
drug-release into the respiratory tract can be
selectively controlled, according to the lipid
20 composition of the liposomes encapsulating the drug.
        The invention includes a method for moderating
the initial (short-term) and extended (long-term)
drug-level effects of a drug administered by
inhalation. The drug is provided in a form in which it.
25 is predomiantly entrapped in the liposomes of a liposome
suspension, and the suspension is aerosolized in a form
suitable for inhalation. According to one aspect of the
invention, the sequestering of the drug predominantly in
a slow-release, liposome-encapsulated form largely
30 reduces or eliminates unwanted drug-spiking effects due
to rapid systemic uptake of the administered drug. The
system thus allows greater amounts of drug to be
administered at a single dose, with fewer side effects.
In the system, the free drug provides a loading dose,

and the liposomes-encapsulated drug provides for
sustained release delivery.

According to another feature of the invention,
the liposomes are formulated to produce a selected rate
5   of release of entrapped drug, for use in achieving
controlled systemic levels of the drug. The drug
release half lives, as measured in vitro may range from
a half hour or less, for liposomes whose phospholipid
acyl chain components are relatively short and/or
10 unsaturated, to six days or more, for liposomes whose
phospholipid acyl chain components are relatively long
and/or saturated.

The system of the invention includes a
suspension of liposomes containing a drug predominantly
15 in entrapped form, and a device for aerosolizing a
preselected quantity of the suspension, in a form
suitable for inhalation.

Two embodiments of the system are designed for
administration of water-soluble drugs, particularly
20 liposome-permeable drugs. In the first, the liposomes
are prepared in an aqueous, paste-like suspension
containing more than 50% encapsulated water, and
therefore, more than 50% encapsulated drug after drug
equilibration between inner liposomal and outer bulk
25 phase compartments of the paste. The paste is mixed
with at least 1-2 volumes of a delivery medium within a
mixing chamber, and aerosolized before drug diffusion
across the liposomal barrier substantially reduces the
percent of liposome-encapsulated drug. The delivery
30 medium may be an aqueous medium, in which case the
diluted liposomes can be aerosolized by pneumatic or
ultrasonic energy. Alternatively, the paste may be
diluted with a fluorocarbon propellent delivery medium,
for use in a self-contained aerosol device.

In a second general embodiment, the liposome suspension in the system is composed of drug-containing liposomes dispersed in a fluorocarbon solvent. The percent liposome-encapsulated drug, which exists in an

5   equilibrated state in the suspension, is relatively high due to the greater partitioning of the drug in the encapsulated aqueous compartment than in the external non-aqueous fluorocarbon medium. The liposomes may be suspended in the fluorocarbon either in dried,

10   particulate form, or as an emulsion of aqueous liposome paste particles. The paste, as just noted, contains the drug predominantly in liposome-encapsulated form. The liposome/propellent suspension can be delivered in metered dose form from a conventional self-contained

15   nebulizer.

These and other objects and features of the present invention will become more fully apparent when the following detailed description of the invention is read in conjunction with the accompanying drawings.

20

Detailed Description of the Drawings

Figure 1 is a plot showing percent protection against acetylcholine-induced bronchoconstriction in an animal following administration by inhalation of free

25   metaproteranol sulfate (MPS)(open squares) or MPS-encapsulated in liposomes having relatively long (closed circles) or relatively short (open circles) in vitro drug efflux half lives;

Figure 2 is a plot showing percent increase in

30   heart rate in an animal following administration of MPS, as in Figure 1.

Figures 3A and 3B are plots showing percent increase in heart rate in a subject following treatment with free MPS (open squares in the figures), and MPS

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encapsulated in liposomes with relatively high (3A) and low (3B) rates of drug release; and

Figure 4 shows plasma concentrations of MPS in a subject following administration of free drug (open
5   squares) and drug encapsulated in each of the Figure 3 liposome formulations.

Detailed Description of the Invention

10 I. Liposome Suspension

The liposome suspension of the invention is formulated to contain (a) a selected drug predominantly in liposome-entrapped form, and (b) a lipid composition designed to produce a desired drug-release rate when the
15 suspension is admisistered to the respiratory tract. Section IA below considers the relationship between liposome bilayer components and drug-release rates. Section IB describes methods for preparing liposomes containing entrapped drugs. Methods of forming liposome
20 suspensions containing a drug in predominantly liposome-entrapped form are discussed in Sections IC-IE.

IA. Lipid Components and Drug Release Rates

In studies conducted in support of the present.
25 invention and reported in Examples I-III, the effects of lipid acyl chain length and degree of unsaturation, lipid charge, and presence of sterol on in vitro drug efflux rates from liposomes were examined. Of factors which were studied, the most important is acyl chain
30 length and the degree of unsaturation in the phospholipid(s) forming the liposomes. The study reported in Example I shows that the in vitro drug efflux half life (a measure of the time required for one-half of the liposome-entrapped drug to be released

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from the liposomes on incubation _in vitro_) can vary over
a hundredfold or more, according to acyl chain length
and degree of unsaturation. In forty different
phosphatidylcholine (PC) lipids and lipid mixtures which
5 were studied, increasing either acyl chain length, or
the degree of saturation of phospholipid acyl chains,
led to greater drug efflux half lives, with the most
significant increases being observed when the liposomes
included a significant proportion of lipids whose
10 transition temperature (T_c) are above the temperature
at which the efflux half lives are measured, e.g., 37°C.

Studies on the effect of lipid charge on drug
release rates, reported in Example II, indicate that the
addition of a negatively charged lipid, such as
15 phosphatidyl glycerol (PG), at a mole ratio of about
10%, produces a slight to moderate increase in efflux
half life. The lipid-charge effect is dependent
somewhat on the degree of saturation and chain length in
the charged and uncharged lipids used in forming the
20 liposomes, with the charged effect producing a greater
increase in drug efflux rate where the liposomes are
formed of predominantly shorter and/or unsaturated
lipids, and producing less effect in the case of
longer-chain and/or saturated lipids.

25 The effect of sterol, either cholesterol or the
negatively charged cholesterol, hemisuccinate, on drug
efflux from liposomes is considered in Example III. A
first study involved liposomes formed with one of eight
PC lipids or lipid mixtures and cholesterol, at a
30 PC/cholesterol mole ratio of 60:40. In general,
cholesterol was found to have a moderating effect on the
acyl composition of the phospholipid, slightly
increasing drug efflux half lives in liposomes
containing predominantly unsaturated and shorter-chain

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length PCs, and producing a significant decrease in the
drug efflux half lives in liposomes containing
predominantly longer-chain and more saturated
phospholipids. This moderating effect may be due to the
5 known effect of cholesterol in decreasing the fluidity
of unsaturated membranes (which would produce greater
efflux half lives) and increasing the fluidity of
saturated membranes (which would produce shorter half
lives).

10   A negatively charged sterol, such as
cholesterol hemisuccinate, when formulated with
uncharged phospholipid(s), appears to have little effect
on drug efflux rates over that observed in liposomes
formed from uncharged phospholipids and cholesterol
15 (Example III). Surprisingly, however, the drug efflux
half lives observed in liposomes composed of uncharged
phospholipids and cholesterol are enhanced significantly
by the addition of negatively charged phospholipid (10
mole percent PG). As seen from the data presented in
20 Table 5 of Example III, in nearly all of the PC mixtures
which were studied, the addition of 10 mole percent PG
to liposomes also containing PC (50 mole percent) and
cholesterol (40 mole percent) enhanced the drug efflux
half life more than twofold, and in some cases more than
25 threefold over those observed for liposomes composed of
PC and cholesterol only. The combination of a neutral
phospholipid, such as PC, a charged phospholipid, such
as PG, and cholesterol appears particularly advantageous
in achieving a range of drug half lives, as measured at
30 37°C, of between about 2 and 24 hours.
          The lipids forming the liposomes may, of
course, include phospholipids and sterols other than the
model lipids used in the above-mentioned studies, and
may also include other types of lipids, such as

glycolipids and sphingolipids, which are compatible with liposome formation. A list of lipids which are used commonly in liposome formation is given on page 471 of reference 2. The liposomes may also be formulated to

5 include various types of drug-protective or lipid-protective agents, such as the antoxidant α-tocopherol, which was typically included at a 1.0 mole percent in the liposomes described herein.

Another factor in the design of liposomes

10 having a selected drug efflux rate is the interaction of the drug with the liposome bilayer(s). The studies reported in Examples I-III involve a water-soluble drug, metaproterenol sulfate (MPS), which is entrapped in liposomes predominantly in encapsulated form, i.e., in

15 the aqueous vesicular space of the liposomes. For more lipophilic drugs, which tend to partition in the lipid bilayer phase of the liposome, the relative effects of acyl chain lengths and saturation, sterol content, and lipid charge density may produce somewhat different

20 effects on drug efflux rate. The procedures described in Examples I-III for determining the effect of various lipid-composition parameters on drug efflux half lives are recommended for determining lipid-composition effects on efflux of other selected lipid-soluble or

25 water-soluble drugs.

I.B. Liposome Preparation

The liposomes may be prepared by a variety of techniques, such as those detailed in reference 2. The

30 choice of the liposome preparation method will depend, in part, on the nature of the drug to be entrapped. As defined herein, "drug" is intended to include any pharmacologically active agent which has a site of action in the respiratory tract or is therapeutically

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active when it is taken up systemically from the
respiratory tract. Such drugs may include antibiotics,
peptide hormones, enzymes, enzyme inhibitors, anti-tumor
agents, bronchodilators, allergens, antihistamines, and
5   biogenic compounds such as steroids and prostaglandins.

For purposes of discussion, three general
classes of drugs will be considered. The first class
are water-soluble, liposome-permeable drugs which are
characterized by a tendency to partition preferentially
10   into the aqueous compartments of the liposome
suspension, and to equilibrate, over time, between the
inner lipsomal spaces and outer bulk phase of the
suspension. The time required for equilibration is, of
course, related to the drug-release rate of the
15   liposomes, and will therefore vary according to the
lipid composition of the liposomes. As seen above,
drug-release half lives of between a few hours and days
are typical. Also, each drug will have its own
characteristic release rate, depending on drug size,
20   charge, and ability to interact with the liposome
membrane. Representative drugs in this class include
terbutaline, albuterol, atropine methyl nitrate,
cromolyn sodium, propranalol, flunoisolide, ibuprofin,
gentamycin, tobermycin, pentamidine, penicillin,
25   theophylline, bleomycin, etoposide, captoprel, n-acetyl
cysteine, and verapamil.

A second general class of drugs are those which
are water-soluble, but liposome-impermeable. For the
most part, these are peptide or protein molecules, such
30   as peptide hormones, enzymes, enzyme inhibitors,
apolipoproteins, and higher molecular weight
carbohydrates are characterized by long-term stability
of encapsulation. Representative compounds in this
class include calcitonin, atriopeptin, α-1

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antitrypsin (protease inhibitor), interferon, oxytocin, vasopressin, insulin, interleukin-2, superoxide dismutase, tissue plasminogen activator (TPA), plasma factor 8, epidermal growth factor, tumor necrosis
5   factor, lung surfactant protein, and lipocortin.

In the third class of drugs are lipophilic molecules which tend to partition into the lipid bilayer phase of the liposomes, and which are therefore associated with the liposomes predominantly in a
10 membrane-entrapped form. The drugs in this class are defined by an oil/water partition coefficient, as measured in a standard oil/water mixture such as octanol/water, of greater than 1 and preferably greater than about 5. Representative drugs include
15 prostaglandins, amphotericin B, progesterone, isosorbide dinatrate, testosterone, nitroglycerin, estradiol, doxorubicin, beclomethasone and esters, vitamin E, cortisone, dexamethasone and esters, DPPC/DPPG phophoslipids, and betamethasone valerete.
20   One preferred method for preparing drug-containing liposomes is the reverse phase evaporation method described in reference 3 and in U.S. Patent No. 4,235,871. In this method, a solution of liposome-forming lipids is mixed with a smaller volume
25 of an aqueous medium, and the mixture is dispersed to form a water-in-oil emulsion. The drug to be entrapped is added either to the lipid solution or aqueous medium. After removing the lipid solvent by evaporation, the resulting gel is converted to
30 liposomes, with an encapsulation efficiency, for a water-soluble drug, of up to 50%. The reverse phase evaporation vesicles (REVs) have typical average sizes between about 2-4 microns and are predominantly oligolamellar, that is, contain one or a few lipid

bilayer shells. The oligolamellar nature of the
vesicles may facilitate drug efflux and thus contribute
to a lower efflux half live for an encapsulated drug.

A simple lipid-hydration procedure for
5   producing multilamellar vesicles (MLVs) may be preferred
where high drug encapsulation efficiency is not
desired. In this procedure, a mixture of
liposome-forming lipids dissolved in a suitable solvent
is evaporated in a vessel to form a thin film, which is
10   then covered by an aqueous solution of the drug. The
lipid film hydrates to form MLVs, typically with sizes
between about 0.1 to 10 microns. As in the REV method,
the drug to be encapsulated is added either to the
initial lipids or the the hydrating medium, depending on
15   its solubility in water. The percent of total drug
material which can be encapsulated in the MLVs,
calculated as the ratio of encapsulated drug to total
drug used in vesicle preparation, is typically between
about 5-20% for water-soluble drugs. As seen in
20   Examples I-III, encapsulation efficiency is dependent to
a degree on lipid composition.

Following liposome preparation, the dispersion
can be treated by extrusion to reduce overall liposome
size and size heterogeneity. Reducing liposome
25   particle size may be important in achieving efficient
aerosolization of the liposomes, and in maximizing drug
deposition in a desired portion of the repiratory
tract. Experiments conducted in support of the present
invention indicate that liposomes contained in an
30   aqueous suspension may be aerosolized efficiently in the
form of liposome-containing aqueous particle mist, where
the liposomes are predominantly less than about 1 micron
in diameter. In terms of targeting the liposomes to a
particular region of the respiratory tract, it is

advantageous to produce liposomes whose sizes are compatible with a desired size range of mist particles. For example, mist particle sizes of greater than about 5 microns would favor deposition in the upper

5   respiratory-tract regions, and particle sizes of between about 0.5-1.5 microns would favor deposition in lower pulmonary regions of the respiratory tract. Generally liposomes having particle sizes less than about 1 micron are compatible with a wide range of mist particle sizes,

10 e.g., from 1-10 microns. As will be seen below, where the liposomes are aerosolized in the form of an aqueous mist, the actual sizes of particles carrying the liposomes may be much greater than that of the liposomes themselves, and thus actual liposome size becomes less

15 crucial to targeting.

One effective sizing method involves extruding an aqueous suspension of the liposomes through a polycarbonate membrane having a selected uniform pore size, typically 0.2, 0.4, 0.6, 0.8 or 1 microns

20 (reference 3). The pore size of the membrane corresponds roughly to the largest sizes of liposomes produced by extrusion through that membrane, particularly where the preparation is extruded two or more times through the same membrane. This method of

25 liposome sizing is used in preparing the liposomes described in the several examples below. A more recent method involves extrusion through an asymetric ceramic filter. The method is detailed in co-owned U.S. patent application for Liposome Extrusion Method, Serial #

30 829,710, filed February 13, 1986.

The liposomes may be further treated to remove unencapsulated drugs and/or other solute material. Conventional separation techniques, such as centrifugation, diafiltration, and molecular-sieve

chromatography are suitable. The separation step should
be carried out after sizing, since the sizing procedure
itself can lead to liposome rupture and release of
encapsulated drug. It will be appreciated that free
5   drug removal will be necessary to remove water-soluble,
liposome-impermeable drugs from the liposome
dispersion. In the case of a lipophilic or
liposome-permeable drug, the relative percent of free
drug in a liposome suspension can be reduced by
10 concentrating the liposomes, according to methods
described in Section IC.

   IC. Liposome Suspension: Impermeable Water-Soluble Drugs
            According to an important feature of the
15 invention, the liposome suspension which is administered
to respiratory tract contains the therapeutic drug of
interest predominantly in a liposome-entrapped form. By
this is meant that at least about 50%, and preferably at
least about 70% of the drug, is contained in a
20 liposome-associated form, i.e., either encapsulated or
membrane-entrapped form. For water-soluble,
liposome-impermeable drugs, the suspension may be a
dilute liposome dispersion in which only about 5%-30% of
the total aqueous volume is encapsulated within the
25 liposomes.
            A dilute suspension of liposomes is readily
prepared, as above, by forming liposomes in the presence
of the drug, sizing if desired, then removing free drug
from the suspension. The final concentration of
30 encapsulated drug should preferably be such that the
liposomes aqueous spaces are iso- or hypo-osmotic with
respect to the environment of the respiratory tract, to
prevent liposome damage by swelling at the delivery
site. The final concentration of liposomes in the

suspension is adjusted to provide a selected quantity of
liposome-encapsulated drug in a given volume of
aerosolized spray. For example, where a maximum amount
of drug is to be delivered in a given suspension volume,
5   the concentration of encapsulated drug should be close
to isotonicity, and the suspension concentrated to
between about 20%-30% encapsulated aqueous volume, i.e.,
the highest liposome concentration which allows direct
aerosolization.

10

ID. Liposome Suspension: Permeable Water-Soluble Drugs
            Where the water-soluble drug is also freely
permeable across the liposomal barrier, it is necessary
to prepare the suspension in a form in which the drug is
15 confined predominantly to the liposomes, after complete
equilibration of the drug between the inner liposomal
and outer bulk phase compartments of the suspension.
This condition is achieved, according to the one
embodiment of the invention, in an aqueous liposome
20 paste in which the encapsulated volume of aqueous medium
is greater than about 50% and preferably about 70-75% of
the total suspension volume, reflecting the approximate
percentage of liposome encapsulated drug.
            In a preferred method of forming the paste, a
25 relatively dilute liposome dispersion, prepared as
above, is concentrated by ultrafiltration, and more
preferably, tangential-flow ultrafiltration. An
multi-filter apparatus for carrying out the filtration
process has been described in co-owned patent
30 application for "Liposome Concentrate and Method", filed
May 7, 1986. Briefly, the dispersion is recyled
continuously through a frame-and-plate type filtration
device having one or more filtration units, each
composed of a central filtrate screen and a pair a

filters supported on the upper and lower sides of
screen. The dispersion is pumped under pressure across
both membranes, and aqueous medium is drawn though the
filters across a pressure gradient of typically about
5 5-25 psi, with higher gradients being required as the
recycled material becomes more concentrated. The
material is recylcled through the system until a desired
liposome concentration, which can be as high as 70-75%
liposomes, expressed in percentage of
10 liposome-encapsulated volume, is achieved. The
ultrafiltration membranes used in the device may be
conventional membranes, such as polysulfone, nylon, or
cellulose acetate membranes which have a size cutoff of
between about 10,000-100,000 daltons.
15   Other concentration methods, such as drying
and centrifugation to pellet liposomes, may also be
used to form the liposome concentrate of the invention,
although ultrafiltration is preferred, because of
advantages of sterility, large-volume capacity, and
20 relatively mild conditions to which the liposomes are
exposed.
          In another general embodiment, the liposome
paste from above is emulsified in a propellant solvent,
to form a double emulsion of liposomes suspended in
25 concentrated form within aqueous emulsion particles
which are themselves suspended in the propellant
solvent.
          The propellant solvent is one in which (a)
liposomes can be suspended without damaging the lipid
30 integrity of the particles, and (b) the drug has a
relatively low partition coefficient with respect to
water, such that the bulk of the drug remains associated
with the liposomes. Example V below examines the
stability of liposome emulsions in six fluorocarbon

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propellants. The propellants which were tested are
"Freon 11" (CCl_3F), "Freon 12" (CCl_2F_2), "Freon
22" ($CHClF_2$), "Freon 113" (CCl_2FCClF_2), "Freon
114" ($CClF_2CClF_2$), and "Freon 115" ($CClF_2CF_3$).

5 Three different formulations of liposomes were tested,
and all showed good stability in Freon 113, 114, and
115. Best stability was achieved with liposomes
containing unsaturated acyl-chain phospholipids . It is
noted here that Freon 114 and 115 can be mixed in

10 proportions which give a proper vapor pressure at room
temperature for an aerosol canister. Dimethylether,
another well-known propellant solvent, may be added to a
fluorocarbon propellant to decrease solvent density, but
the amount added must be carefully monitored to prevent

15 solvent damage to the liposomes.

To form the double emusion, the liposome paste
from above is added to the propellant solvent, at a
final volume concentration of between about 5%-30%
preferably. The mixture may further contain an

20 emulsifier, such as Tween-80, to enhance the stability
of the emulsion. Vigorous mixing, for example, by
sonication, is used to produce the final emulsion.

In another embodiment, the liposomes suspension
is prepared by dispersing dried liposomes, in powdered

25 or particulate form, in a propellant solvent of the type
just considered. The dried liposomes preferably include
a bulking agent, which is added to improve the
formulation in several ways: The agent improves the
milling properties of the dried material, and also

30 increase the density of the particles, to give better
dispersion in relatively dense fluorocarbon
propellants. The bulking agent also acts to shield the
liposomes and the encapsulated drug from the propellant
and provides greater particle cohesion in the suspended

form. There is also evidence that the bulking agent
reduces membrane damage on drying and dehydrating, so
that less free drug is produced when the dried liposomes
rehydrate in the moist environment of the lung.

5   Finally, since the hydroscopic nature of the bulking
agent improves hydration during passage through the
airways. Preferred stabilizing agents include a variety
of mono- or disaccharides, such as glucose, mannitol,
trehalose, sucrose, and lactose, which are tolerated

10 well in the respiratory tract.

        Methods for forming liposomes containing such
bulking agents in encapsulated form are described in
co-owned patent application for "Stabilized
Liposome/Amphotericin Composition and Method, Serial No.

15 781,395, filed October 17, 1985. In one preparation
method, a solution of vesicle-forming lipids are added
in an organic solvent to bulking agent particles, and
the lipid solvent is evaporated, depositing the lipids
as a coating on the particles. The preparation method

20 now follows the above MLV procedures, with addition of
hydrating medium producing MLVs with dissolved bulking
agent contained in the encapsulated and outer bulk phase
aqueous compartments. Non-encapsulated agent may be
removed before dehydrating the liposomes.

25   The liposome dispersion or paste is dehydrated
by known techniques, which may include lyophilization,
or spray drying under vaccuum conditions. After drying,
the liposomes are particlized by milling or the like, to
a final size of less than about 5 microns, and

30 preferably, less than about 1 microns. The liposome
powder is added to the propellant solvent to a final
concentration preferably between about 1-10% by weight.

D. Liposome Suspension: Lipid-Soluble Drugs

     The liposome suspension for use with lipophilic
drugs is an aqueous suspension whose lipid
concentration is at least sufficient to ensure that 50%
5 or more and preferably 70% or more of the drug is in the
lipid (liposome bilayer) phase of the suspension, and
within the liposomes. The liposome concentration
required will depend on the partition coefficient of the
drug, as well as other factors mentioned above. If the
10 drug partition constant is only slightly greater than 1,
the suspension would have to be concentrated to a
paste-like consistency in which about half or more of
the total aqueous volume is liposome encapsulated. With
a partition coefficient of about 10, a much more dilute
15 aqueous dispersion would be possible, Of course, the
extent of allowed dilution is also limited by the need
to entrap an adequate drug dose in a fairly small amount
of aerosolized material.

20 II. Aerosolizing Liposomes

     The present inhalation system also includes a
device for aerosolizing the liposome suspension in a
form suitable for inhalation. These devices are
discussed below in relation to the three general types
25 of lipsome suspensions to be nebulized.

IIA. Dilute Liposome Suspensions

     Pneumatic nebulizers which are designed for
atomizing aqueous sample are commercially available, and
30 operate according to well-known procedures, such as
those outlined in Example IV. Typically, the nebulizing
operation is carried out at a pressure of about 5-15
psi, and the aqueous particles formed are typically in
the range of about 2-6 microns. The device may be

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controlled to produce a measured quantity of aerosolized
liposomes, according to known operational variables
(reference 4).

Because the aerosolization procedure may result
5   in mechanical disruption of a liposome suspension, it
may be important to establish that the nebulizing
process does not significantly affect liposome integrity
and size. The effect of aerosolization by a pneumatic
nebulizer on liposome particle size and loss of
10 encapsulated material is reported in Example IV. Here
four liposome compositions having _in vitro_ drug efflux
half lives ranging from 206 minutes to 5256 minutes, and
each having an original particle size distribution
between about 0.180-0.300 microns, were examined for
15 size change and loss of encapsulated material after
aerosolization at 5 psi. As reported in Table 7 of
Example IV, no change in liposome size or loss of
encapsulated material was observed for three of the
preparations, and only a slight loss of encapsulated
20 material and moderate increase in size was observed for
liposomes with the lowest drug efflux half life.

Another device suitable for aerosolizing an
aqueous suspension of liposomes, and preferably a
relatively dilute suspension containing less than about
25 25%-30% encapsulated aqueous volume, uses ultrasonic
energy to break up a carrier fluid into a fine mist of
aqueous particles. The ultrasonic nebulizer device has
been found to produce a liposome aerosol mist whose
particle sizes are about the same as those formed by a
30 compressed air nebulizer, i.e., between about 2-6
microns. The effect on liposome size and retention of
encapsulated material by ultrasonic aerosolization was
examined in a study similar to the one just described,
and also reported in Example IV below. The results of

that study, summarized in Table 8 in Example IV, are
consistent with the earlier study, showing that the
aerosolizing process has substantially no effect on
liposome size or release of encapsulated material,
5 except a relatively minor effect on the liposomes
composed predominantly of short, unsaturated acyl chain
lipids.

IIB. Liposome Paste Suspensions
10   For aerosolizing a liposome paste of the type
used for delivery of a water-soluble, liposome-permeable
drug, the paste is first mixed with a carrier solvent,
to form a diluted dispersion which can be aerosolized.
The carrier solvent may be an aqueous medium, in which
15 case the paste is diluted to a form suitable for
spraying, such as by a pneumatic or ultrasonic
nebulizer. The amount of diluent added is sufficient to
dilute the paste to a sprayable dispersion, preferably
containing less than about 30% total encapsulated
20 volume. Assuming the paste has an initial encapsulated
volume of 70-75% of the total paste volume, it can be
appreciated that a given volume of the paste must be
diluted with at least one and two volumes of diluent.
          Alternatively, the carrier solvent may be a
25 propellant solvent, which when mixed with the liposome
paste, forms either a paste-in-propellant emulsion, of
the type described above in Section ID, or a foamed
paste mixture formed by introducing pressurized
propellant into the paste. The volume ratio of paste to
30 propellant is preferably between about 10%-30%, giving
an emulsion or foam composition which can be readily
aerosolized.
          A suitable aerosolizing device for the making
and spraying the emulsion includes (1) separate chambers

for holding the paste and carrier vehicle liquids, (2) a
mixing chamber where metered amounts of the two liquids
are mixed, and (3) means for aerosolizing the contents
of the mixing chamber. Dispensing devices designed for
5 storing two different liquids in isolation from each
other, and for intermittant dispensing of the liquids in
contact with each other are known, as disclosed, for
example, in U.S. patent # 3,325,056. Where the device
operates to mix the paste with an aqueous carrier
10 medium, the pneumatic or ultrasonic energy used to
produce aerosolization can also be used to effect rapid
and complete mixing of the two fluids.. The releasing
means includes valve structure communicating with the
mixing chamber, to allow release of the contents of the
15 mixing chamber through a nozzle. Here it is noted that
the mixing and spraying steps are carried out in
relatively quick succession, before appreciable leakage
of encapsulated drug into the aqueous carrier vehicle
can occur, i.e., while the drug is still predominantly
20 in liposome-encapsulated form.

    In the case of a self-contained nebulizer
containing a propellant carrier fluid, the two fluids
may be mixed under conditions which cause rapid breakup
of the paste by gassification or foaming, just prior to
25 ejecting the gassified mixture through a nozzle to
achieve aerosolization. Alternatively, the device can
operated to entrain the paste in emulsion form in the
liquid propellant, just prior to ejecting the mixture.
The releasing means is a valve structure which
30 communicates with the mixing chamber, to allow release
of the self-pressurized contents of the chamber through
a nozzle. The propellant solvent is preferably a
fluorocarbon or fluuorocarbon/diethylether mixture, such
as discussed above, which does not itself cause

appreciable liposome disruption. However, since the
liposomes are in contact with solvent for only a brief
mixing and spraying period, problems related to liposome
disruption and drug partitioning across the liposomal
5   barrier are less serious than where the bulk phase of
the liposome suspension is itself a propellant solvent,
i.e., where the liposomes are initially formulated in a
propellant solvent.

10   IIC.   Preformed Liposome/Propellant Suspensions
          Valved container devices for aerosolizing
particulate material suspended in a propellant solvent
are well known. These devices generally operate by
supplying a particle suspension to a metering chamber,
15   and forcing the contents of the chamber through a nozzle
under pressure, to aerosolize a metered quantity of the
suspension. In the present invention, the
particle/propellant suspension is either a suspension of
dried particulate liposomes, or a suspension of liposome
20   paste emulsion particles, prepared as in Section ID
above. With both types of emulsions, particle
separation from the propellant on standing may require
that the container be shaken vigorously before use, to
resuspend the liposomes uniformly in the solvent. It is
25   noted that almost all self-contained aerosol devices
require vigorous shaking before use.

III.   Therapeutic Applications
          A variety of respiratory tract conditions and
30   diseases, including nasal congestion, asthma and other
bronchospasm conditions, nasal inflammation, and
vascular headaches, may be treated by drug inhalation
therapy according to the present invention.
Representative drugs which are useful in the treatment

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of respiratory tract conditions and diseases are listed
in reference 1, and include nasal decongestants such a
propylhexedrine and methylhexylamine; respiratory
stimulants such as epinephrine, isoproteranol and
5 metaproterenol, and isoetharine; corticosteroids such as
dexamethasone and beclomethasone; and other medications
such as cromolyn, sodium acetyl cysteine, and ergotamine
tartrate.

In preparing liposomes for targeting a drug to
10 the respiratory tract, the liposomes are formulated to
produce a selected _in vitro_ drug efflux rate, preferably
measured at about 37°C, for achieving a controlled drug
release at the target site in the respiratory tract. It
has been seen in Section IA how lipid-composition
15 factors can be varied to achieve _in vitro_ drug half
lives ranging from as short as half an hour or less up
to several days. According to another important aspect
of the invention, the liposome composition can be varied
selectively to moderate unwanted systemic side effects
20 observed when the drug is taken up systemically from its
site of deposition in the pulmonary region of the
respiratory tract. Generally, the same lipid
composition factors which lead to longer drug efflux
half lives are effective in reducing systemic drug
25 levels which are observed shortly after administration
of the drug by inhalation. For example, to produce
greater reduction in initial systemic drug level
effects, the liposomes may be formulated with a greater
percentage of long-chain and/or saturated acyl chain
30 phospholipids.

The application of the method and system of the
invention for the treatment of bronchoconstriction is
illustrated in Example VI below. The animals studied
were treated by inhalation with MPS in free form or

encapsulated in one of two different-composition
liposomes--one having an _in vitro_ drug efflux half life
of 250 minutes and the other, an efflux half life of
about 1150 minutes.   Both liposome formulations were
5 delivered in aqueous aerosol particles in which the drug
was contained predominantly in encapsulated form, and
the amount of MPS delivered was about the same for both
liposome-encapsulated and free MPS.   After drug
administration, the degree of protection against
10 bronchoconstriction provoked by aerosols of
acetylcholine solution was measured approximately every
15 minutes for a four hour period.   Typical results for
two animals, representing the average of three runs for
both free drug and the two liposome-encapsulated drug
15 formulations, are shown in Figure 1.   The average
percent protection values are indicated by open squares
for the free drug, by closed circles for the long
half-life (1150 minutes) liposome formulation, and by
open circles for the short half-life (250 minutes)
20 liposome formulation.   As seen from the data, both free
and liposome-encapsulated drug provided about the same
degree of protection as the free drug.
        The above MPS inhalation treatment also
illustrates the ability of liposome-encapsulated MPS to
25 protect the animal against tachycardia, a relatively
severe systemic side effect seen when MPS is
administered by inhalation in free form.   The effect of
free drug and liposome-encapsulated drug on percent
increase in heart rate during the four hour period after
30 MPS administration by inhalation was also measured.
Typical results, representing the average of three
experiments for each of free drug and the two
liposome-encapsulated drug formulations, are shown in
Figure 2.   As in Figure 1, the data corresponding to

free drug is indicated by open squares, the long
half-life liposome formulation, by closed circles, and
the short half-life liposomes by open circles. As seen
from the figure, the free drug produced an approximately
5 90% increase in heart rate within about 5 minutes of
drug administration, and this heart rate gradually
subsided over the four hour observation period to about
a 50% level.

By contrast, treatment with the same
10 concentration of drug, but contained in liposome
formulation, produced an initial increase in heart rate
observed in the first half hour after drug
administration, of between 20%-30%. This rate remained
substantially constant over the four hour period,
15 evidencing the fact that a relatively small amount of
drug is delivered in utilizable form initially, but is
delivered to the site of absorption at a relatively
constant rate over an extended drug-delivery period.

The short half-life liposome formulation (open
20 circles in Figure 2) also protected against high initial
heart rate increase and showed substantially constant
systemic drug levels over the four-hour monitoring
period. Interestingly, the measured heart rate for the
short half-life formulation was uniformly about two
25 times higher than that of the long half-life formulation
throughout the monitoring period.

A similar group of tests were performed to
measure the therapeutic and systemic side effects
produced by delivering liposome-encapsulated MPS to
30 anesthetized guinea pigs. The studies involved the four
liposomes compositions: (1) EPG/EPG/cholesterol/α-T
(5:1:1:0.1), which has a MPS release half life of 250
minutes (Example III); (2) DSPC/DSPG/cholesterol/α-T
(5:1:1:0.1), which has a MPS release half life of 1148

minutes (Example III); (3) DPPC/DPPG/cholesterol/α-T
(5:1:1:0.1), which has a MPS release half life of 941
minutes (Example III); and (4) DPPC/DPPG/α-T
(9:1:0.1), which has a MPS release half life of 5256
minutes (Example II). Each composition contained 1%
MPS, predominantly in encapsulated form.

      The animals were administered one of the four
liposome compositions, or saline (as a control) or free
drug. The animals in each test group were stabilized
for 15 minutes after anesthesia, then administered a
controlled volume of aerosol by intubation for a two
minute period. At, 15, 45, 90, and 105 minutes after
aerosol delivery, the animals recived a dose of
histamine, over a fifteen second interval, to cause
transient, asthma-like bronchoconstriction. The animals
were monitored for airway resistance, dynamic
compliance, heart rate, and blood plasma levels of MPS
at 30 minute intervals over a two hour period after
aerosol administration. The airway resistance and
dynamic compliance measurements indicate the degree of
protection provided by the MPS treatment against the
histamine-induced bronchoconstriction. Formulation 1,
with a relatively high release rate (low drug release
half life) was about as effective as free drug in
preventing increased airway resistance and dynamic
compliance, whereas formulations 2 and 3, both with
intermediate drug-release rates, showed therapeutic
effects which were intermediate between free drug and
saline control. Interestingly, formulation 4, which has
the lowest drug release rate _in vitro_, was similar to
formulation 1 and free drug in its protection against
airway resistance and dynamic compliance increases. The
explanation for this anomaly may be that the DPPC/DPPG
lipids in the formulation 4 liposomes are dominant

phopholipids in the respiratory tract, and may therefore
exchange phospholipids with or become integrated into
the lipid membranes at the target site relatively
rapidly, with accompanying release of drugs. This
5 supposition is supported by data on systemic drug
effect, discussed below, which indicates that
formulation 4 liposomes give systemic drug levels which
are intermediate between those of formulation 1 and and
those of formulations 2 and 3. It is also possible that
10 the DPPG and DPPG lipids in the formulation 4 liposomes
are therapeutically effective, independent of the
release of MPS.

The percent increase in heart rate provides, as
in the earlier study, a measure of the systemic action
15 of the drug. As seen in Figures 3A and 3B, free MPS
(open squares in the figures), produces a large initial
increase in heart rate, which then falls slowly over the
next 90 minutes to a level about 20% above normal. This
percent increase is much smaller when the drug is
20 administered in encapsulated form, either in formulation
1 liposomes (open circles in Figure 3A), or in
formulation 4 liposomes (closed circles in Figure 3B).
Also consistent with the earlier study, the more stable
liposome formulation (4) was substantially more
25 effective in reducing the unwanted heart stimulation
side effect than the formulation 1 liposomes. Although
not shown in the figures, the two intermediate
formulations 2 and 3 gave heart rate increases,
reflecting systemic drug levels, which were both
30 appreciably lower than seen with formulation 4.
The effect of liposomes in reducing MPS levels
in the bloodstream after drug delivery to the lungs is
more dramtically apparent from the plasma MPS
concentration levels shown in Figure 4. With free drug,

plasma drug levels were nearly 80 ng/ml initially, with
a slow decline over a two-hour period to about 20
ng/ml. By contrast, with all of the liposome
formulations, plasma drug levels were maintained at a
5 susbstantially constant level below about 10 ng/ml
throughout the test period.

The above data demonstrate two important
features of the invention. The first is the ability of
liposomes to moderate the initial and extended
10 drug-level effects of a drug administered by
inhalation. That is, the liposomes function to
"flatten" the drug-release curve seen for free drug by
reducing initial drug-level effects and maintaining
elevated drug levels over an extended period. The
15 second feature is the ability to control or regulate
drug release rates _in vivo_ using liposomes having a
suitably selected _in vitro_ drug efflux rate. As seen in
the above system, the selected rate of drug release can
be varied up to about two fold by suitable selection of
20 liposomes whose _in vitro_ drug efflux rates vary over
about a 4-5 fold range.

In formulating liposomes for use in inhalation
treatment, it is of course necessary to achieve a
therapeutically effective dose of drug during the period
25 of sustained drug release, and for this reason,
liposomes having very long drug efflux half lives may be
suboptimal therapeutically. In the bronchoconstriction
treatment described above, the comparable therapeutic
effect of liposome-encapsulated MPS, with the respect to
30 free drug, suggests that even the long half-life
liposomes are functioning to release the drug at a
concentration which is optimal or near-optimal for
therapeutic effect.

According to an important aspect of the
invention, the inhalation system and method just
described are advantageous for delivering drugs
systemically, as well as for local respiratory tract
5   delivery. The controlled drug-release rates for a
sytemic-acting water-soluble drug (MPS) are seen above.
Lipid soluble drugs, which are contained predominantly
in the lipid bilayer region of liposomes, gradually
become associated with endogenous lung lipids, with
10 liposome deposition in the lower respiratory tract, and
in this form, the drugs can traverse the blood-gas
barrier to enter the pulmonary circulation via
concentration-dependent diffusion.
Delivery of drugs in a predominantly
15 liposome-associate form solves a variety of problems
encountered when the drug is administered in free or
predomianantly free form. In addition to the drug
moderating and sustained-release features seen above,
the liposomes act to protect the drug from oxidation and
20 protect the respiratory tract from potentially
irritating drugs, particularly those which, because of
solubility properties, must be administered in
micronized form.
In one application for intrapulmonary drug
25 delivery, α-1-protease inhibitor is delivered to the
pulmonary interstitium in liposome-encapsulated form to
stem the development of pulmonary emphysema. The
liposomes act to protect the protease inhibitor's
tertiary structure from oxidation, and facilitate its
30 transport across the pulmonary cell membranes.
An example of a specific application for
systemic drug delivery is the encapsulation and delivery
of nitroglycerin, a coronary vessel dilator used to
relieve the symptoms of angina pectoris. The drug

formulation typically will contain about 30%-40% free
drug, which can be rapidly absorbed by the pulmonary
blood flow and transported directly to the heart, its
primary site of action, to provide immediate relief from
5   the chest pain associated with angina. The remaining
60%-70% or more of the liposome-encapsulated drug is
released slowly, at a rate controlled by liposome
composition, to afford prolonged coronary vessel
dilation, and thus relief from chest pain for an
10   extended period.

         Oxytocin, a peptide hormone that induces and
augments the strength of uterine muscle contractions
during labor, can be formulated and delivered in a
manner similar to that described for nitroglycerine. It
15   is currently delivered by intravenous infusion, a
process that requires placement and maintenance of a
venous cannula, a sometimes difficult procedure that
limits the patient movement and posture. Aerosols of
liposome-oxytocin formulation would provide immediate
20   and sustained delivery to the systemic circulation,
similar to that provided by IV infusion, without
restricting patient motion.

         From the foregoing, it can be appreciated how
various objects and features of the invention are met. ·
25   The method of the invention allows for administration of
lipid- or water-soluble drugs, under conditions which
produce a controlled release of drug at the target
site. The controlled release of drug may serve the
purpose of extending the time interval between drug
30   administrations, moderating short and long-term cyclical
variation in drug levels, either at the site of
deposition or systemic drug levels, and/or substantially
reducing unwanted systemic side effects.

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The liposomes used in the method and system of the invention can be formulated to achieve a desired controlled drug release rate, by formulating the liposomes to produce a selected _in vitro_ drug efflux

5 rate.

The inhalation method and system are compatible with a variety of different aerosolization procedures which allow for the administration by inhalation of liposomes in various sized aerosol particles and form,

10 ranging from rather large aqueous particles to relatively small powdered liposome preparations.

Toxicity studies conducted in support of the invention showed no adverse effects of liposomes administered by inhalation. Under the conditions of the

15 study acute (four hour) exposure to a single dose of aerosolized liposomes), there were no statistically significant differences between a phosphate-buffered saline (blank) and two liposome-exposed mouse populations in body weights, fresh excised lung weights,

20 and light-microscope histopathologic findings.

The following examples are intended to illustrate various features and uses of the present invention, but are in no way intended to limit the scope

25 thereof.

Example I
Effect of Lipid Chain Length and Saturation
on Drug Efflux

30 Metaproteranol sulfate (MPS) was obtained from Vinchem Inc. (Chatham, NJ); α-tocopherol (α-T), from Sigma Chemical Co. (St. Louis, MO); and ^{14}-C sucrose, from Amersham Co. (Arlington Hts, IL). Egg phosphatidyl choline (EPC), soy phosphatidyl choline (SPC),

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hydrogenated egg phosphatidyl choline (HEPC),
hydrogenated soy phosphatidyl choline (HSPC), dioleoyl
phosphatidyl choline (DOPC), dimyristoyl phosphatidyl
choline (DMPC), dipalmitoyl phosphatidyl choline (DPPC),
5 and distearoyl phosphatidyl choline (DSPC), were
obtained from Avanti Polar Lipids (Birmingham, AL).
The 8 PC lipids have the transition temperatures
(T_c) and fatty acyl chain compositions given in Table 1
below, where the lipids having greater acyl chain
10 lengths and/or degrees of saturation are arranged in
descending order.

Table 1

PC	T_c (C°)	Fatty Acyl Composition
SPC	−15 to −7	16:0(12%) 18:2(73%)
DOPC	−22	18:1
EPC	−15 to −7	16:0(42%) 18:1(28%) 18:2(16%)
DMPC	23	14:0
DPPC	41	16:0
HEPC	55 to 65	16:0(36%) 18:0(60%)
HSPC	55 to 65	16:0(13%) 18:0(87%)
DSPC	55	18:0

25   For each of the 8 PC lipids, multilamellar
vesicles (MLVs) were prepared by dissolving 350 µmole
of the selected PC lipid with 1 mole percent of α-T in
chloroform. The dissolved lipid was evaporated to
dryness under vacuum in a round-bottom flask to form a
30 lipid film on the flask walls. A 5 ml solution
containing 20 mg/ml MPS and 1.1 x 10^7 cpm
^{14}C-sucrose in phosphate-buffered saline (PBS), pH
7.2, 290 mOsm, was added to the flask to cover the
film. At the end of a 2-hour hydration period, the

liposomes (MLVs) which formed were extruded successively
two times each through a 0.4 and 0.2 micron uniform
pore-size polycarbonate filter (Bio-Rad; Richmond, CA).
Unencapsulated MPS and sucrose were removed by passage
5   of the extruded MLVs through a Sephadex G-75
gel-exclusion column. The percent encapsulation of MPS
ranged from a low of about 0% for DMPC MLVs to a high of
21% for DPPC MLVs (Table 2). Encapsulation of
^{14}C-sucrose ranged from a low of 0.2% for DMPC MLVs to
10 a high of 18% for the DPPC MLVs.

        To measure leakage of encapsulated solutes from
the MLV preparations, each preparation was incubated at
37°C for a period of up to 42 hours. Timed aliquots
taken periodically during the 42-hour incubation period
15 were passed through a gel-exclusion column, and the
lipid, MPS, and sucrose present in the liposome peak
were assayed by conventional methods. The loss of
lipid-associated drug during the course of the
incubation was used to determine the drug efflux half
20 life ($t_{1/2}$) for each preparation, calculated as the
time required for the drug/lipid ratio to fall by
one-half. The calculated efflux half lives, expressed
in minutes, are shown in Table 2 below.

25

30

Table 2

Encapsulation

PC/α-T (10:0.1)	% MPS Encapsulated	$t_{1/2}$ MPS (minutes)
SPC	6	22
DOPC	4	41
EPC	10	48
DMPC	0	—
DPPC	21	574
HEPC	5	6426
HSPC	8	2175
DSPC	13	6366

As seen from data in Table 2, the rate of
efflux of drugs from the MLVs can be selectively
increased from a half life of about 22 minutes to one
showing no appreciable leakage over a 42-hour period, by
increasing the length and degree of saturation of the
acyl chains in the phospholipids making up the
liposomes. A comparison of the half-life data in Table
2 with the transition temperature data in Table 1 shows
that each of the MLV preparations having a relatively
high efflux half life (greater than 500 minutes) is
composed of a PC whose transition temperature is above
the temperature at which the liposomes were incubated.
No appreciable loss of the relatively impermeable
^{14}C-sucrose from the liposomes was detected over the
42 hour incubation period, indicating that the observed
drug efflux is not due to liposome breakdown.

Example II
Effect of Charged Lipids on Drug Efflux

Egg phosphatidyl glycerol (EPG), dioleoyl
phosphatidyl glycerol (DOPC), and distearoyl
5 phosphatidyl choline (DSPC) were obtained from Avanti
Polar Lipids (Birmingham, AL).

Liposomes (MLVs) containing the lipid
composition indicated at the left in Table 3 below, were
prepared substantially as described in Example I,
10 starting with initial concentrations of 312 μmole of
the selected PC, 35 μmole of the selected PG, and 3.5
μmole α-T. For each lipid composition, the dried
film was hydrated in 5 ml of the MPS/sucrose solution
described in Example I, and the liposomes were sized by
15 extrusion through polycarbonate filters and separated
from non-encapsulated material by molecular-sieve
chromatography, as above. The percentages of
encapsulated MPS are shown in the middle column in Table
3. A comparison of the encapsulation data from Tables 2
20 and 3 shows that the presence of 10% PG generally
increased drug encapsulation efficiencies. This effect
was also observed for ^{14}C-sucrose encapsulation.

The eight MLV fractions were each incubated at
37°C for 42 hours, and the drug efflux half lives
25 determined from the measured loss of drug at several
intervals over the 42-hour incubation period. The
calculated efflux rate half lives are shown at the right
in Table 3.

30

Table 3

PC/PG/α-T (9:1:0.1)	% MPS Encapsulated	$t_{1/2}$ (minutes)
SPC/EPG	14	92
DOPC/DOPG	13	289
EPC/EPG	7	151
DPPC/EPG	16	110
DPPC/DPPG	23	5256
HEPC/EPG	15	526
HSPC/EPG	14	856
DSPC/DSPG	20	14100

The addition of 10 mole percent PG to the liposome/lipid mixture had one of two effects relative to PCs alone (Example I). For the shorter, more unsaturated PC acyl chains, an increase in half life for MPS leakage was seen as a result of the negative charge imparted to the bilayer by PG. With the saturated, longer chain length lipids, the more important factor seems to be the degree of acyl chain unsaturation of the added PG--for example, in the case of EPG, decreasing the overall membrane saturation and thus decreasing measured half lives for MPS leakage. Where a PG derivative of the same saturation chain length as the PC was used (DPPG/DSPG), similar or longer half-lives than with PC alone resulted. As in Example I, no appreciable loss of ^{14}C-sucrose was observed in any of the preparations.

Example III
Effect of Cholesterol on Drug Efflux

Eight liposome MLV preparations containing one of the eight PCs shown at the left in Table 4,

cholesterol (CH), and α-T in a molar ratio of 6:4:0.1, and containing encapsulated MPS and ^{14}C-labeled sucrose, were prepared as in Example I. The MLV preparations, after extrusion through 0.4 and 0.2

5   polycarbonate membranes and treatment with molecular-sieve chromatography to remove unencapsulated material, gave the percent encapsulation of MPS indicated in the middle column in Table 4.

     The eight MLV fractions were each incubated for

10   a 42-hour period to determine drug efflux half lives   in accordance with the methods described above. The calculated half lives are shown at the right in Table 4.

Table 4

15

PC/CH/α-T (6:4:0.1)	% Encapsulated MPS	$t_{1/2}$ (minutes)
SPC/CH	8	103
DOPC/CH	9	313
EPC/CH	10	206
DMPC/CH	9	201
DPPC/CH	11	146
HEPC/CH	11	220
HSPC/CH	10	330
DSPC/CH	13	421

     In the presence of 40 mole percent cholesterol, drug efflux half lives associated with unsaturated, shorter-chain lipids were longer than those obtained for

30   PC alone. For the more saturated, longer chain-length lipids, 40 mole percent cholesterol decreased half lives significantly over that observed for PC alone.

     The effect of cholesterol and PG was examined in a similar type of study using MLVs composed of 50

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mole percent of a selected PC, 10 mole percent of a
selected PG, 40 mole percent of cholesterol, and 0.1
mole percent α-T, as indicated at the left in Table
5. The drug encapsulation data for the eight MLV
5   preparations and the corresponding drug efflux half
lives are shown at the middle and right columns,
respectively, in Table 5. As seen, the presence of 10
mole percent PG significantly increased the drug efflux
half lives of each PC lipid with respect to the Table 4
10   lipid composition containing PC and cholesterol only.

Table 5

PC/PG/CH/α-T (5:1:1:0.1)	% Encapsulated MPS	$t_{1/2}$ (minutes)
SPC/EPG/CH	10	326
DOPC/DOPG/CH	11	235
EPC/EPG/CH	12	250
DMPC/DMPG/CH	10	591
DPPC/DPPG/CH	12	941
HEPC/EPG/CH	13	334
HSPC/EPG/CH	12	553
DSPC/DSPG/CH	27	1148

25   Finally, eight liposome preparations containing
one of the PC lipids indicated at the left in Table 6
(60 mole percent); cholesterol hemisuccinate, a
negatively charged sterol (40 mole percent); and 0.1
mole percent α-T were prepared as above. CHHS was
30   obtained from Sigma Chem Co. (St. Louis, MO). The eight
preparations gave the encapsulation efficiencies and
drug efflux half lives indicated in the middle and right
columns, respectively, in Table 6 below. As seen, the
presence of 40 mole percent cholesterol hemisuccinate

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gave uniformly high encapsulation efficiencies. The
half lives are generally somewhat greater than those
observed with the corresponding liposome preparations
involving 40 mole percent cholesterol (Table 4 above),
5 but significantly less than the corresponding lipid
preparations containing 40 mole percent cholesterol plus
10 mole percent PG.

Table 6

10

PC/CHHS/α-T (9:1:0.1)	% Encapsulated MPS	$t_{1/2}$ (minutes)
SPC/CHHS	19	73
DOPC/CHHS	19	123
EPC/CHHS	24	77
DMPC/CHHS	20	129
DPPC/CHHS	18	245
HEPC/CHHS	20	601
HSPC/CHHS	19	856
DSPC/CHHS	23	768

Example IV

Aerosolizing Aqueous Liposome Suspensions

This section examines the effect on liposome
25 integrity, as judged by loss of encapsulated material
and change in liposome size, of aerosolizing an aqueous
liposome suspension.

Four liposome preparations were tested.
Preparation #1 had a composition EPC/CH/α-T, at a
30 molar ratio of 6:4:0.1; preparation #2, a composition of
DSPC/CH/α-T, at a molar ratio of 6:4:0.1; preparation
#3, a composition of DPPC/DPPG/CH/α-T, at a molar
ratio of 5:1:4:0.1; and preparation #4, a composition of
DPPC/DPPG/α-T, at a molar ratio of 9:1:0.1. The four

preparation represent a wide range of drug-efflux half
lives, measured as in Examples I-III above, as indicated
in Table 7 below. The liposomes were prepared
substantially as described in Example I, except that the
5   aqueous solution used in preparing the liposomes
contained carboxyfluorescein (129 ξmole) rather than
MPS and ^{14}C-labeled sucrose. Each of the four
liposome preparations was extruded two times each
through a 0.4 and 0.2 micron polycarbonate membrane to
10 produce substantially homogeneous particle sizes in the
particle size ranges indicated below in Tables 7 and 8
after the liposome suspensions were separated from
unencapsulated material by extensive dialysis. The
final concentration of liposomes in the aqueous
15 suspension was 70 μmole total lipid per ml PBS.

        For each lipid preparation, liposome size
distribution and percent encapsulation was measured
before aerosolization (B), after aerosolization (A), and
in the suspension which remained in the nebulizer (R).
20 The sizes of liposomes in the suspension were determined
by using a Nicomp Instruments Laser Particle Sizer Model
200 (Hyak-Royco, Menlo Park, CA). The machine was
calibrated periodically with monodisperse latex beads
supplied by the manufacturer for use as external
25 standards.

        The percent encapsulated carboxyfluorescein was
determined according to standard procedures.

        The size and percent encapsulation data for
liposome preparations before and after aerosolization by
30 the pneumatic nebulizer are shown in Table 7 below. The
device used was a De Vilbiss No. 646 Pneumatic Nebulizer
(Somerset, PA), operated at 15 psi. As seen from the
data, aerosolization of the liposomes resulted in only a
small loss of encapsulated material, ranging from about

8% for the liposome preparation having the lowest efflux
half life to about 1% for the material having the
highest efflux half life. No significant change in the
size distribution of liposomes was observed for any of
the four liposome preparations after aerosolization.

Table 7

	Preparation		% Encap.±s.d.(n=3)	Median Lip. size (nm)
10				
	1	B	96.2±0.11	214, 210-218
	$t_{1/2}=$	A	88.5±0.13	375, 315-428
	206 min.	R	95.9±0.04	193, 189-200
15	2	B	95.5±0.20	230, 214-268
	$t_{1/2}=$	A	93.0±0.08	200, 180-240
	421 min.	R	96.7±0.13	200, 190-218
	3	B	97.2±0.63	327, 271-450
20	$t_{1/2}=$	A	93.4±0.04	257, 225-327
	941 min.	R	96.2±0.93	353, 300-400
	4	B	96.1±0.19	214, 193-231
	$t_{1/2}=$	A	95.1±0.11	218, 171-266
25	5256 min.	R	96.5±0.02	225, 200-276

The effect on the integrity of the four
liposome preparations of aerosolization by an ultrasonic
nebulizer was also examined. The nebulizer used was a
DeVilbiss Pulmasonic Model 25 Ultrasonic Nebulizer
(Somerset, PA), operated at a power mode of 3 kHz. The
percent carboxyfluorescein encapsulation and size
distribution, measured as above, are shown in Table 8
below for each of the four preparations, measured before

(B) and after (A) aerosolization and for material
remaining in the nebulizer (R). The results are
substantially identical with the results obtained for
the same preparations aerosolized by a compressed-air
device.

Table 8

Preparation		% Encap.±s.d.(n=3)	Median Lip. size (nm)
B = Before		A = After	R = Remainder
	B	95.6±0.10	316, 285-375
1	A	89.4±0.65	261, 250-279
	R	94.9±0.45	207, 188-222
	B	98.6±0.08	240, 185-300
2	A	96.8±0.07	214, 200-240
	R	97.7±0.53	163, 150-189
	B	98.1±0.09	257, 240-270
3	A	96.0±0.09	254, 244-270
	R	97.2±0.10	240, 171-600
	B	98.5±0.02	333, 310-375
4	A	96.2±0.01	220, 204-231
	R	96.8±0.03	220, 208-230

Example V

Liposome Stability in Fluorocarbon Propellants

This section examines the stability of three
liposome preparations in chlorofluorocarbon propellant
solvents. The first liposome preparation, referred to
as formulation 1, contained DPPC/DPPG/α-T at a molar

ratio of 9:1:0.1; the second preparation, designated
formulation 2, contained EPC/EPG/α-T at a molar ratio
of 9:1:0.1; and the third preparation, designated
formulation 3, was composed of EPC/EPG/CH/α-T at a

5   molar ratio of 5:1:4:0.1. The liposomes were prepared
substantially as described in Example I, except that
aqueous solution used in forming the MLVs contained
bovine serum albumin (BSA) and 1 μM carboxyfluorescein
(CF). MLV preparations extruded twice through each of a

10   0.4 and a 0.2 micron polycarbonate membrane were
pelleted by centrifugation to remove unencapsulated
material, and resuspended in PBS to a final lipid
concentration of about 20 μmol/ml. The six propellant
solvents used are "Freon 11" (CCl_3F), "Freon 12"

15   (CCl_2F_2), "Freon 22" ($CHClF_2$), "Freon 113"
(CCl_2FCClF_2), "Freon 114" ($CClF_2CClF_2$), and
"Freon 115" ($CClF_2CF_3$).
        An aqueous suspension of each preparation in
PBS (3 ml) was added to each of the 6 fluorocarbon

20   solvents (10 ml) in a pressurized vial, and the vials
were shaken on a mechanical shaker for 24 hours to
maintain a water-and-oil emulsion. After the shaking
period, the bulk liquid phases in each vial were allowed
to reform by settling, and the upper aqueous phase was

25   removed by aspiration. The liposomes in the aqueous
phase were pelleted by centrifugation, and resuspended
to the original lipid concentration in PBS. A portion
of the resuspended liposomes was examined for particle
size distribution, substantially as described in Example

30   IV. A control sample, which was not exposed to a
fluorocarbon but was otherwise handled identically to
the other samples, showed a size distribution of between
about 180 and 250 nm. The various samples which had
been exposed to the six propellant solvents generally

fell into two size ranges: those having sizes
substantially identical to the control liposome sizes,
i.e., between about 150 and 300 microns; and those
having predominantly greater sized (400-500 microns or
5 larger). The results, seen in Table 9 below, show that
two of three preparations were stable in "Freon 12",
that all three were stable in "Freon 114" and "Freon
115", and that MLVs containing largely saturated lipids
(formulation 1) are generally more stable in
10 fluorochlorocarbon propellants than those having
predominantly unsaturated and/or shorter-chain
phospholipids (formulations 2 and 3).

     The pelleted and resuspended liposomes were
also examined for release of BSA, representative of a
15 relatively large molecular weight marker, and
carboxyfluorescein, representative of a relatively small
encapsulated marker. For each test, an aliquot of the
resuspended liposomes were assayed by conventional
methods for the appropriate marker. The marker
20 concentrations are expressed in Table 9 in terms of
amount marker per μmole of total lipid in the sample
assayed (μg of BSA, μmole of CF). "ND" in the table
means "not detectable ".

25

30

Table 9

Freon Propellant	Formulation 1			Formulation 2			Formulation 3		
	Size	BSA	CF	Size	BSA	CF	Size	BSA	CF
Control	191±26	14.3	11.4	248±84	7.1	3.8	248±60	8.2	5.9
11	171±35	13.5	12.6	495±293	ND	ND	560±355	ND	ND
12	177±44	11.4	10.3	231±76	8.2	3.2	703±400	4.0	1.9
22	1060±570	ND	ND	475±260	5.9	1.8	772±430	12.0	1.1
113	157±43	10.1	8.8	254±60	ND	ND	160±60	ND	ND
114	188±40	11.2	9.3	236±65	9.7	3.7	220±50	7.8	8.0
115	181±40	13.2	11.1	188±50	8.6	3.2	240±55	8.0	5.8

ND=not detectable

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As seen from the data in the table, the loss of
encapsulated material correlates generally with
increased liposome size, both reflecting instability of
liposomes in the particular fluorochlorocarbon solvent..

5   However, both preparations 2 and 3 showed substantial
loss of encapsulated material without significant size
change when exposed to "Freon 113"..

Example VI

10   ### Treatment of Bronchoconstriction

This example examines the therapeutic effect of
free and liposome-encapsulated metaproteranol sulfate on
bronchoconstriction induced by aerosols of acetylcholine
(ACH).

15   Two mongrel dogs, a 20 kg male and a 30 kg
female, were anaesthetized with an initial i.v. bolus
injection of sodium thiopental (10 mg/kg body weight)
and sodium pentabarbital (20 mg/kg body weight) and
subsequent boluses of sodium thiopental every thirty

20   minutes, or as needed, to maintain the dog in an
anaesthetized state over a four hour period. The
animals were intubated with a cuffed endotracheal tube,
placed on a heat-exchange pad and artificially
ventilated by a constant-volume respirator with room air

25   at a frequency of 30 cycles/minute. An esophageal
balloon catheter was introduced into the esophagus,
connected to one port of a differential pressure
transducer, and positioned in the mid-thorax to produce
a maximal negative pressure inflection during

30   inspiration. Air flow was monitored by a
pneumotachograph connected to the endotracheal tube and
the respirator and connected to a differential pressure
transducer. Transpulmonary pressure was monitored by a
differential pressure transducer, one port connected to

a side arm of the tracheal tube and the other end
connected to the esophageal balloon catheter. The flow
and pressure signals were displayed continuously on an
oscilloscope.

5   Liposome preparations having a lipid
composition of DSPC/DSPG/CH/α-T at a molar ratio of
5:1:4:0.1 (Preparation 1) and EPC/EPG/CH/α-T at the
same molar ratio (Preparation 2), each encapsulating
metaproteranol sulfate at a final 2% drug concentration,
10 were prepared as in Example III. As seen from Table 5
above, Preparation 1 has a drug efflux half life of
about 1150 minutes, and Preparation 2, of about 250
minutes. Free MPS was administered as a 2% solution in
PBS. Liquid aerosols of acetylcholine solutions
15 sufficient to produce transient bronchoconstriction, as
reflected by a 2-10 fold increase in total pulmonary
resistance were administered via the endotracheal tube
before, and at roughly 15 minute intervals following,
aerosol administration of either free or
20 liposome-encapsulated metaproteranol, each in doses
corresponding to a total of about 2.4 mgs MPS. All
aerosols, including the acetylcholine aerosol, free-drug
aerosol, and liposome aerosol, were generated with a
pneumatic nebulizer (Bird #158, Somerset, PA), according
25 to manufacturer's specifications, at 15 psi. Aerosol
particle sizing performed with a Mercer Cascade Impactor
(InTox Products, NM), indicated mass aerodynamic aerosol
particle diameters within 2-6 microns.
          The effects of the free-drug and liposome
30 aerosols on bronchoconstriction were measured
approximately every fifteen minutes for four hours after
initial drug administration. The experiment was
repeated 3 times for each free and liposome-encapsulated
drug formulation for each animal, and the values

averaged. The results obtained for dog #1 are shown in
Figure 1, and are expressed in terms of percent
protection from ACH-induced bronchoconstriction. Each
point on the graph represents the mean ± standard
5 error of the mean from results obtained in three
experiments. As seen from the figure, free drug (open
squares), Preparation 1 (closed circles), and
Preparation 2 (open circles) all showed substantially
the same degree of protection against ACH-induced
10 bronchoconstriction. If any trend is discernible from
the data, it is that the liposome-encapsulated drug
appears to be more effective in the period between 2-4
hours after drug administration. Similar results were
obtained for the other dog treated.
15

Example VII
Systemic Drug Delivery by Inhalation
The systemic drug levels of MPS, when
administered by inhalation in either free or
20 liposome-encapsulated form, were examined by monitoring
the change in heart rate produced by administration of
the drug according to the experimental protocol
described above in Example VI. For each animal study
conducted in Example VI, heart rate measurements were
25 taken approximately every fifteen minutes in the
four-hour period following drug administration. The
heart rate was measured continuously on an oscilloscope
via skin-needle leads and recorded intermittently on a
polygraph. The data obtained for each of three runs on
30 each of the three drug preparations (free drug and drug
encapsulated in long half-life and short half-life
liposomes) were averaged and plotted as percent increase
over normal resting heart rate. The results for one of
the treated animals are shown in Figure 2. As in Figure

1, each point represents the mean ± standard error of the mean from results obtained in three experiments. As seen in the figure, the free drug produced a more than 90% increase in heart rate within the first five minutes

5 of drug administration, and the measured heart rate declined gradually over the 4-hour course of the measurement period to a level of about a 50% heart rate increase. Preparation 1 (the long half-life liposomes) showed an initial heart rate increase of between about

10 20-30% and remained within that range substantially over the entire monitoring period. Preparation 2 (the short half-life liposomes) gave an initial heart rate increase of about 40%, and this level also remained substantially constant over the four-hour monitoring period.

15 While preferred embodiments of the invention have been described herein, it will be appreciated that various changes and modifications may be made without departing from the invention.

20

25

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IT IS CLAIMED:

1.   A system for administering a drug, at a
controlled drug-release rate, via the respiratory tract,
comprising:
        a suspension of liposomes containing the drug
in predominantly liposome-entrapped form, where the
phospholipid composition of the liposomes is formulated
to produce a selected rate of drug release from
liposomes when administered to the respiratory tract,
and
        a device for aerosolizing a selected quantity of
the suspension, in a form suitable for inhalation.

2.   The system of claim 1, wherein the drug is
a water-soluble, liposome-permeable drug, and the
suspension is an aqueous suspension in which more than
50% of the drug is encapsulated within the liposomes.

3.   The system of claim 2, wherein said
suspension is a liposome paste in which more than 50% of
the aqueous phase is encapsulated within the liposomes,
and said device includes a mixing chamber in which a
measured volume of paste is mixed with at least 1-2
volumes of the delivery medium immediately before
aerosolization.

4.   The system of claim 3, wherein said device
includes separate chambers for holding said paste and
the delivery medium, a mixing chamber where the paste
and delivery medium are mixed, and means for releasing
the contents of the mixing chamber through a nozzle, to
aerosolize such contents.

5.   The system of claim 4, wherein the delivery medium is an aqueous medium, and said releasing means includes valve structure operatively connected to a pneumatic or ultrasonic energy source.

6.   The system of claim 4, wherein the delivery means includes a fluorocarbon propellant, and said releasing means includes valve structure for releasing the contents of the mixing chamber, under pressure, through said nozzle.

7.   The system of claim 6, wherein the propellant is selected from the group consisting of CCl_2F_2, $CClF_2CClF_2$, and $CClF_2CF_3$.

8.   The system of claim 1, wherein the drug is a water-soluble drug, the liposome suspension includes liposomes suspended in a fluorocarbon propellant, and said device includes valve structure for releasing the suspension under pressure generated by the propellant.

9.   The system of claim 8, wherein the propellant is selected from the group consisting of CCl_2F_2, $CClF_2CClF_2$, and $CClF_2CF_3$.

10. The system of claim 8, wherein the liposomes are suspended in dehydrated particulate form in the propellant.

11. The system of claim 8, wherein the liposomes are emulsified and suspended in a concentrated paste-like form as particles in the fluorocarbon propellant.

12. The system of claim 1, wherein the drug is a water-soluble, liposome-permeable drug which has unwanted systemic side effects, and the suspension contains more than about 70% of the drug in

5 liposome-encapsulated form.

13. The system of claim 12, wherein the drug is metaproterenol sulfate or a pharmaceutically acceptable salt thereof.

10

14. The system of claim 1, wherein the rate of drug release from the liposomes when administered to the respiratory tract is proportional to the drug-release rate _in vitro_, and the phospholipid composition of the

15 liposomes is formulated to produce a selected _in vitro_ drug efflux half-life of between about 250 and 1200 minutes.

15. The system of claim 1, for use in

20 administering a water-soluble, liposome-permeable drug selected from the group consisting of terbutaline, albuterol, atropine methyl nitrate, cromolyn sodium, propranalol, flunoisolide, ibuprofin, gentamycin, tobermycin, pentamidine, penicillin, theophylline,

25 bleomycin, etoposide, captoprel, n-acetyl cysteine, and verapamil.

16. The system of claim 1, for use in administering a water-soluble, liposome-impermeable drug

30 selected from the group consisting of calcitonin, atriopeptin, α-1 antitrypsin (protease inhibitor), interferon, vasopressin, insulin, interleukin-2, superoxide dismutase, tissue plasminogen activator (TPA), plasma factor 8, epidermal growth factor, tumor

necrosis factor, heparin, lung surfactant protein, and
lipocortin.

17.   The system of claim 1, for use in
administering a lipid-soluble drug selected from the
group consisting of prostaglandins, amphotericin B,
progesterone, isosorbide dinitrate, testosterone,
nitroglycerin, estradiol, doxorubicin, beclomethasone
and esters, vitamin E, cortisone, dexamethasone and
esters, DPPC/DPPG phospholipids, and betamethasone
valerete.

18.   In the treatment of a condition or disease
which includes the steps of (1) selecting a
water-soluble drug which is active in treating the
condition or disease, and (2) administering the drug by
inhalation to the respiratory tract, a method of
moderating systemic drug-level effects with respect to
those produced when the drug is administered in free
form, and achieving a selected rate of drug release into
the respiratory tract, comprising:
        providing a suspension of liposomes containing
the drug in predominantly liposome-entrapped form, where
the phospholipid composition of the liposomes is
formulated to produce such a selected rate of drug
release from liposomes when administered to the
respiratory tract, and
        aerosolizing the liposomes in a form suitable
for inhalation.

19.   The method of claim 18, wherein the drug
is a bronchodilator, and the amount of drug releasd into
the bloodstream is 20% or less that released into the

bloodstream initially when an equivalent amount of the
drug is administered in free form.

     20.   The method of claim 19, wherein the drug
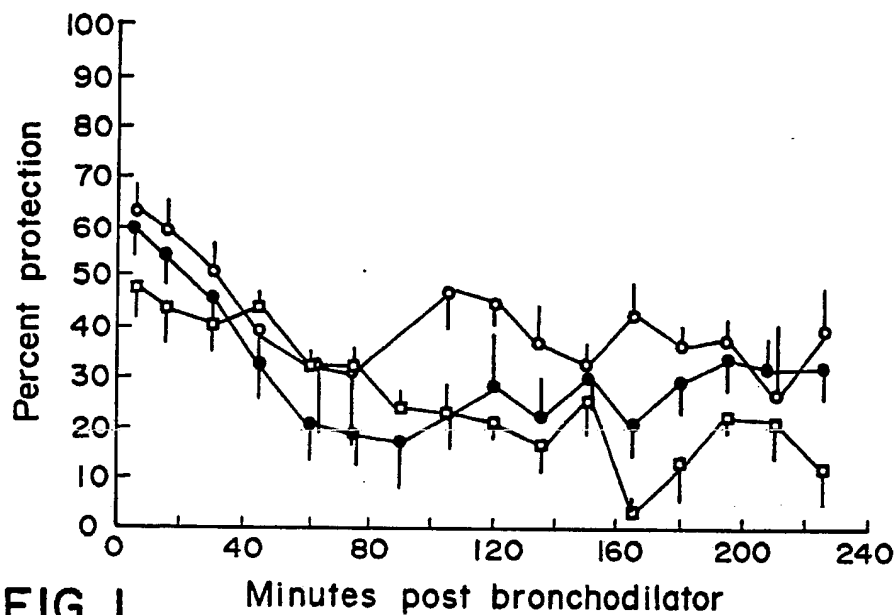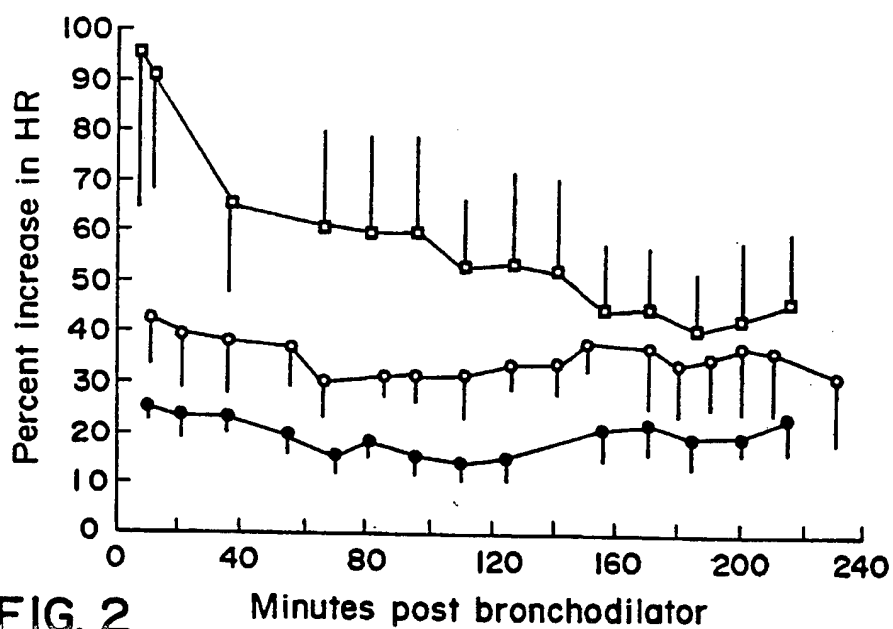is metaproterenol or a pharmacologically acceptable salt
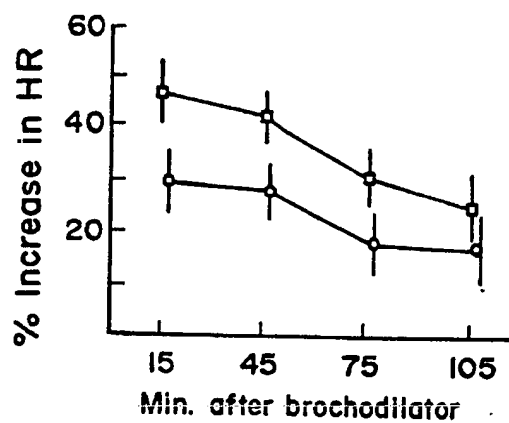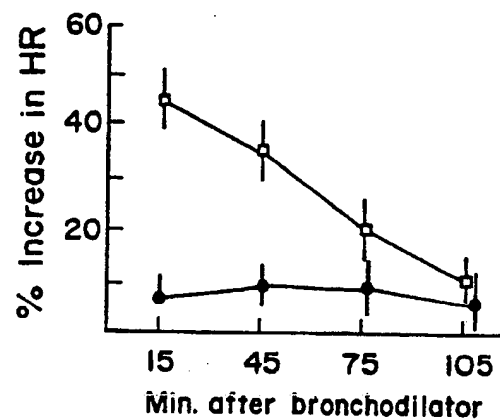thereof.

FIG. I
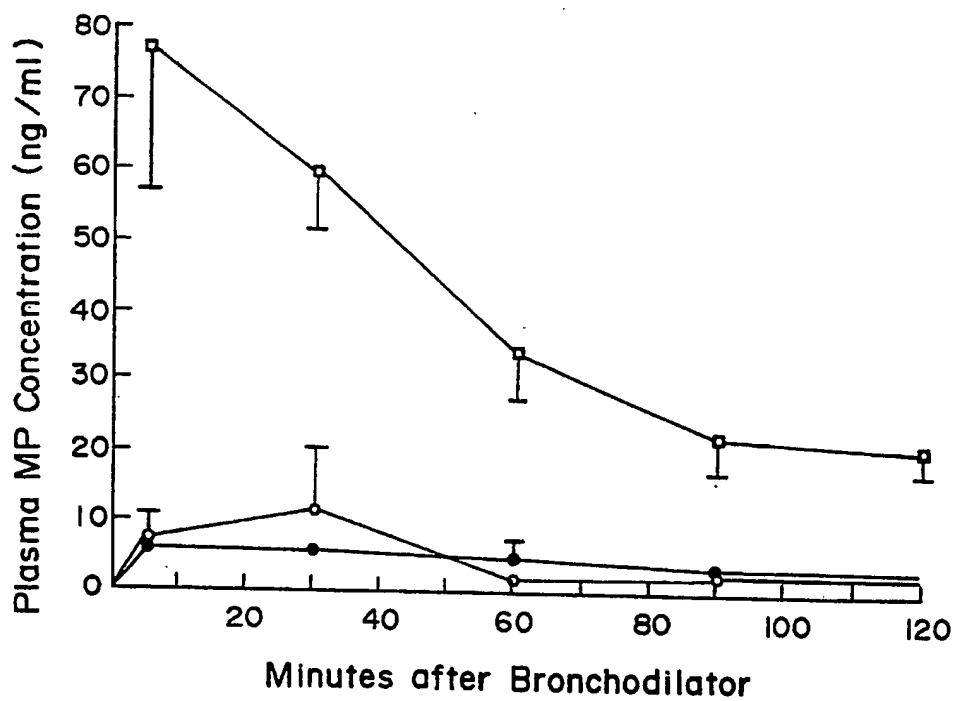

FIG. 2

FIG. 3A

FIG. 3B

FIG. 4

INTERNATIONAL SEARCH REPORT

International Application No PCT/US86/01095

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) 3

According to International Patent Classification (IPC) or to both National Classification and IPC   4

A61K 9/50 A61K 9/42
A61K 31/685

II. FIELDS SEARCHED

Minimum Documentation Searched 4

Classification System	Classification Symbols
U.S.	424/38, 514/78, 937, 938, 943, 958 128/200.23

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched 6

III. DOCUMENTS CONSIDERED TO BE RELEVANT 14

Category *	Citation of Document, 16 with indication, where appropriate, of the relevant passages 17	Relevant to Claim No. 18
Y	US,A, 4,011,864, (GUICHARD), March 15, 15, 1977, see entire document	1-20
Y	US,A, 4,137,914, (WETTERLIN), February 6, 1979, see entire document	1-20
Y	US,A, 4,312,860, (CLEMENTS), January 26, 1982, see entire document	1-20
Y	US,A, 4,414,972, (YOUNG ET AL), November 15, 1983, see entire document	1-20
X Y	EP,B, 84,898, Fisons PLC, August 3, 1983 see entire document	1,12,15,18 2-11,13,14, 16, 17
Y	H.R. Shepard, Aerosols: Science and Technology, Published 1961 by Interscience Publishers, Inc. see pages 17-40, 397-405	1-20,17- 25,29-40

* Special categories of cited documents: 15

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search 2	Date of Mailing of this International Search Report 2
11 August 1986	1 9 AUG 1986
International Searching Authority 1	Signature of Authorized Officer 20
IS/US	Ciro Faraci

Form PCT/ISA/210 (second sheet) (May 1986)